

GE Healthcare

ÄKTAprime™ plus

Operating Instructions

Original instructions



ÄKTA™

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1 Introduction

Purpose of the Operating Instructions

The Operating Instructions provides you with the instructions needed to handle the ÄKTApriime plus system in a safe way.

Prerequisites

In order to operate the system in the way it is intended, the following pre-requisites must be fulfilled:

- You should have a general understanding of how the PC and Windows™ works (if a computer is used).
 - You should understand the concepts of liquid chromatography.
 - You must read and understand the Safety Instructions.
 - The instrument and software should be installed, configured and calibrated according to the Installation Guide.
-

In this chapter

This chapter contains important user information, and a general description of the ÄKTApriime plus system and its intended use.

1.1 Important user information

Read this before using the ÄKTAprime plus system



All users must read the safety instructions in the ÄKTAprime plus User Documentation to fully understand the safe use of the ÄKTAprime plus system, before installing, using, or maintaining the system.

Do not operate the ÄKTAprime plus system in any other way than described in the user documentation. Otherwise, you may be exposed to hazards that can lead to personal injury, and you may cause damage to the equipment.

Intended use

ÄKTAprime plus is a compact liquid chromatography system designed for one-step purification of proteins at laboratory scale.

The ÄKTAprime plus system is intended for research use only, and shall not be used in any clinical procedures, or for diagnostic purposes.

Safety notices

This user documentation contains WARNINGS, CAUTIONS and NOTICES concerning the safe use of the product. See definitions below.

Warnings



WARNING

WARNING indicates a hazardous situation which, if not avoided, could result in death or serious injury. It is important not to proceed until all stated conditions are met and clearly understood.

Cautions



CAUTION

CAUTION indicates a hazardous situation which, if not avoided, could result in minor or moderate injury. It is important not to proceed until all stated conditions are met and clearly understood.

Notices



NOTICE

NOTICE indicates instructions that must be followed to avoid damage to the product or other equipment.

Notes and tips

- Note:** *A Note is used to indicate information that is important for trouble-free and optimal use of the product.*
- TIP:** A tip contains useful information that can improve or optimize your procedures.

Typographical conventions

Software items are identified in the text by ***bold italic*** text. A colon separates menu levels, thus ***File:Open*** refers to the ***Open*** command in the ***File*** menu. Hardware items are identified in the text by **bold** text (e.g., **Power** switch).

1.2 Regulatory information

This section describes the directives and standards that are fulfilled by the ÄKTAprime plus system.

Manufacturing information

The Declaration of Conformity includes the following information:

Requirement	Content
Name and address of manufacturer	GE Healthcare Bio-Sciences AB Björkgatan 30, SE-751 84 Uppsala, Sweden
Name and address of person responsible for Technical File	Peter Löwendahl, Björkgatan 30 SE-751 84 Uppsala, Sweden
Name and ID of notified body	INTERTEK SEMKO AB, NB 0413
Place and date of declaration	Uppsala, Sweden, May 2009
Identity of person authorized to sign DoC	Peter Löwendahl

CE Conformity

This product complies with the European directives listed in the table, by fulfilling the corresponding harmonized standards. A copy of the Declaration of Conformity is available on request.

Directive	Title
2006/42/EC	Machinery Directive (MD)
2006/95/EC	Low Voltage Directive (LVD)
2004/108/EC	ElectroMagnetic Compatibility (EMC) Directive

International standards

This product fulfills the requirements of the following standards:

Standard	Description	Notes
EN 61010-1, IEC 61010-1, UL 61010-1, CAN/CSA-C22.2 No. 61010-1	Safety requirements for electrical equipment for measurement, control, and laboratory use	

Standard	Description	Notes
EN 61326-1	EMC emissions and immunity requirements for electrical equipment for measurement, control and laboratory use	Harmonized with 2004/108/EC
EN-ISO 12100-1, 12100-2	Safety of machinery - Basic concepts, general principles for design	Harmonized with 2006/42/EC
EN-ISO 14121-1, 14121-2	Safety of machinery - Principles of risk assessment	Harmonized with 2006/42/EC

CE Marking

The CE marking and the corresponding Declaration of conformity is valid for the instrument when it is:

- used as a stand-alone unit, or
- connected to other CE marked instruments, or
- connected to other products recommended or described in the user documentation, and
- used in the same state as it was delivered from GE Healthcare, except for alterations described in the user documentation.

The Declaration of conformity is valid only for systems that are marked with the CE-marking.



Regulatory compliance of connected equipment

Any equipment connected to the ÄKTApriime plus system should meet the safety requirements of EN 61010-1/IEC 61010-1, or relevant harmonized standards. Within EU, connected equipment must be CE marked.

1.3 Instrument

Product description

ÄKTApime plus is a compact liquid chromatography system designed for one-step purification of proteins at laboratory scale.

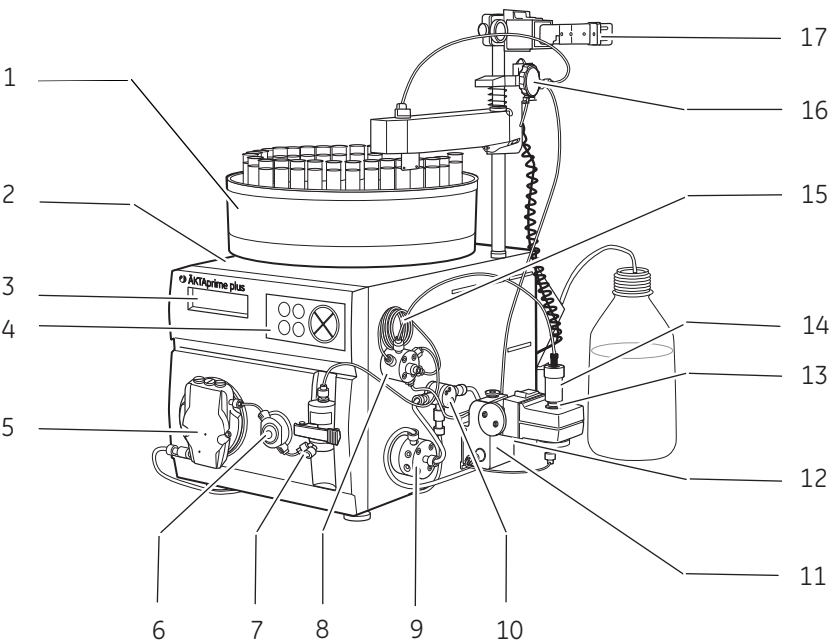


Figure 1.1: The main parts of the instrument.

Part	Function	Part	Function
1	Fraction collector	10	Switch valve
2	Monitor and controller	11	Conductivity cell
3	LCD display	12	Flow restrictor
4	Push buttons	13	UV flow cell
5	Pump	14	Column
6	Pressure sensor	15	Sample loop
7	Mixer	16	Flow diversion valve
8	Injection valve	17	Column holder

Part	Function	Part	Function
9	Buffer valve		

The **Power** switch is located at the rear of the system.

Navigation menu

The system is operated from the push buttons and LCD display at the front panel.

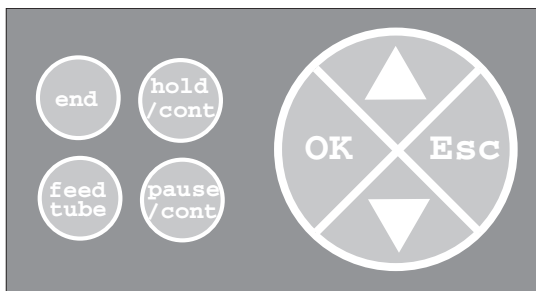


Figure 1.2: Push buttons.

Button	Description
Δ or ∇	Find a specific menu option
OK	Enter a menu.
Esc	Return one menu level.
end	Interrupt method operation before the run is completed. Stop manual operation.
hold /cont	Hold method time or volume and the gradient at the current concentration. Pump and fraction collector continue uninterrupted. Continue the normal method operation.
pause /cont	Pause all operation without ending the method. All functions, including pump and fraction collector, are stopped. Continue the normal method operation.
feed tube	Advance the fraction collector one position.

Basic flow path

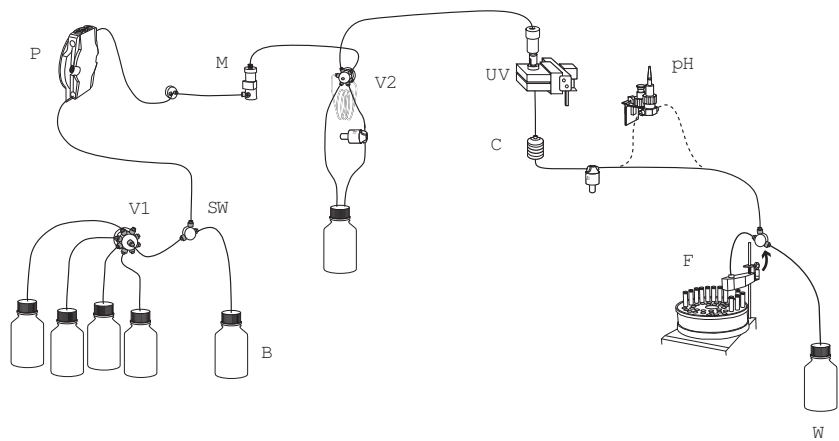


Figure 1.3: Basic flow path.

Stage	Part	Description
1	P, V1	Pump P pumps buffer from a buffer container connected to the buffer valve V1.
2	SW, B	To form a gradient the switch valve (SW) can be used to pull liquid from buffer container (B).
3	M	The mixer (M) mixes the buffers.
4	V2	Sample is applied from the sample loop connected to injection valve (V2) that has been previously filled manually using a syringe.
5	UV, C, pH	From the injection valve, the flow is directed to the column, and then to the UV, Conductivity, and optional pH monitor.
6	F, W	From the monitors, the flow is directed to the Fraction collector F or the Waste W.

1.4 Monitoring and evaluation

PrimeView™ software

PrimeView is a software that allows real time monitoring, evaluation and report generation on an external computer.

For more information about PrimeView evaluation system, see the *PrimeView User Manual* supplied.

Paper chart recorder

It is possible to connect a chart recorder to ÄKTApriime plus to get real time monitoring. For more information see the *ÄKTApriime plus User Manual*.

2 Safety instructions

This chapter describes safety compliance, safety labels, general safety precautions, emergency procedures, power failure and recycling of ÄKTAprime plus system.

2.1 Safety precautions

Introduction

The ÄKTAprime plus instrument is powered by mains voltage and handles pressurized liquids that may be hazardous. Before installing, operating or maintaining the system, you must be aware of the hazards described in this manual. **Follow the instructions provided to avoid personal injury or damage to the equipment.**

The safety precautions in this section are grouped into the following categories:

- General precautions
 - Using flammable liquids
 - Personal protection
 - Installing and moving the instrument
 - System operation
 - Maintenance
-

General precautions

Always follow these General precautions to avoid injury when using the ÄKTAprime plus instrument.



WARNING

Do not operate the ÄKTAprime plus instrument in any other way than described in the ÄKTAprime plus and PrimeView manuals.



WARNING

Operation and user maintenance of the ÄKTApriime plus instrument should be performed by properly trained personnel only.



WARNING

Before connecting a column to the ÄKTApriime plus instrument, read the instructions for use of the column. To avoid exposing the column to excessive pressure, make sure that the pressure limit is set to the specified maximum pressure of the column.



WARNING

Do not use any accessories not supplied or recommended by GE Healthcare.



WARNING

Do not use the ÄKTApriime plus instrument if it is not working properly, nor if it has suffered any damage, for example:

- damage to the power cord or its plug
- damage caused by dropping the equipment
- damage caused by splashing liquid onto it



CAUTION

Waste tubes and containers shall be secured and sealed to prevent accidental spillage.



CAUTION

Make sure waste container is dimensioned for maximum possible volume when the instrument is left unattended.

2 Safety instructions

2.1 Safety precautions



NOTICE

Avoid condensation by letting the unit equilibrate to ambient temperature.

Using flammable liquids

When using flammable liquids with the ÄKTApriime plus instrument, follow these precautions to avoid any risk of fire or explosion.



WARNING

Fire Hazard. Before starting the system, make sure that there is no leakage.



WARNING

A fume hood or similar ventilation system shall be installed when flammable or noxious substances are used.

Personal protection



WARNING

Always use appropriate personal protective equipment during operation and maintenance of ÄKTApriime plus system.



WARNING

Hazardous substances. When using hazardous chemical and biological agents, take all suitable protective measures, such as wearing protective glasses and gloves resistant to the substances used. Follow local and/or national regulations for safe operation and maintenance of the system.



WARNING

Spread of biological agents. The operator has to take all necessary actions to avoid spreading hazardous biological agents in the vicinity of the instrument. The facility should comply with the national code of practice for biosafety.



WARNING

High pressure. The ÄKTAprime plus instrument operates under high pressure. Wear protective glasses at all times.

Installing and moving the instrument



WARNING

Supply voltage. Make sure that the supply voltage at the wall outlet corresponds to the marking on the instrument, before connecting the power cord.



WARNING

Protective ground. The ÄKTAprime plus instrument must always be connected to a grounded power outlet.



WARNING

Power cord. Only use power cords delivered or approved by GE Healthcare.

2 Safety instructions

2.1 Safety precautions



WARNING

Access to power switch and power cord. Do not block the rear and side panel of the instrument. The **Power** switch must always be easy to access. The power cord must always be easy to disconnect.



WARNING

Installing the computer. The computer should be installed and used according to the instructions provided by the manufacturer of the computer.



NOTICE

Disconnect power. To prevent equipment damage, always disconnect power from the ÄKTAprius plus instrument before an instrument module is removed or installed, or a cable is connected or disconnected.

System operation



WARNING

Hazardous chemicals during run. When using hazardous chemicals, run **System CIP** and **Column CIP** to flush the entire system tubing with distilled water, before service and maintenance.



WARNING

Hazardous biological agents during run. When using hazardous biological agents, run **System CIP** and **Column CIP** to flush the entire system tubing with bacteriostatic solution (e.g. NaOH) followed by a neutral buffer and finally distilled water, before service and maintenance.



WARNING

There must always be a sample loop connected to ports 2 and 6 of the injection valve. This is to prevent liquid spraying out of the ports when switching the valve. This is especially dangerous if hazardous chemicals are used.



CAUTION

Hazardous chemicals in UV flow cell. Make sure that the entire flow cell has been flushed thoroughly with bacteriostatic solution, for example NaOH, and distilled water, before service and maintenance.



NOTICE

If the ÄKTApriime plus is kept in a cold room, cold cabinet or similar, keep the system switched on in order to minimize the risk of condensation. (The UV lamp can be turned off to save lamp life time when the system is not in use.)



NOTICE

When switching off the cold cabinet, make sure that you also switch off the ÄKTApriime plus system and leave the door to the cold cabinet open to avoid overheating.

Maintenance



WARNING

Electrical shock hazard. All repairs should be done by service personnel authorized by GE Healthcare. Do not open any covers or replace parts unless specifically stated in the user documentation.

2 Safety instructions

2.1 Safety precautions



WARNING

Disconnect power. Always disconnect power from the instrument before replacing any component on the instrument, unless stated otherwise in the user documentation.



WARNING

Hazardous chemicals during maintenance. When using hazardous chemicals for system or column cleaning, wash the system or columns with a neutral solution in the last phase or step.



WARNING

Do not perform any type of maintenance work while the system is powered electrically or when the piping system is pressurized. Note that the piping system can be pressurized even when the system is closed down.



WARNING

Only spare parts that are approved or supplied by GE Healthcare may be used for maintaining or servicing the system.



WARNING

Make sure that the piping system is completely leakage free before performing any CIP on the system.



WARNING

NaOH is corrosive and therefore dangerous to health. When using hazardous chemicals, avoid spillage and wear protective glasses and other suitable personal protective equipment.



WARNING

After assembly, the piping system must be tested for leakage at maximum pressure for continued protection against injury risks due to fluid jets, burst pipes or explosive atmosphere.



WARNING

Before disassembly, check that there is no pressure in the piping system.



WARNING

Disconnect power. Always disconnect power from the instrument before replacing fuses.



WARNING

Decontaminate the equipment before decommissioning to ensure that hazardous residues are removed.



CAUTION

The system uses high intensity ultra-violet light. Do not remove the UV lamp while the system is running. Before replacing a UV lamp, ensure that the lamp cable is disconnected from the rear of the system to prevent injury to eyes.

If the mercury lamp is broken, make sure that all mercury is removed and disposed according to national and local environmental regulations.



NOTICE

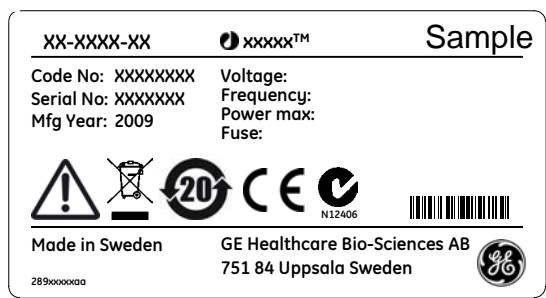
Cleaning. Keep the instrument dry and clean. Wipe regularly with a soft damp tissue and, if necessary, a mild cleaning agent. Let the instrument dry completely before use.

2.2 Labels

This section describes the safety labels and labels concerning hazardous substances that are attached to the ÄKTAprime plus instrument. For information about marking of the computer equipment, refer to the manufacturer's instructions.

Labels on the instrument



The illustration below shows an example of the identification label that is attached to the ÄKTAprime plus instrument.



Symbols used in safety labels

Label	Meaning
	Warning! Read the user documentation before using the system. Do not open any covers or replace parts unless specifically stated in the user documentation.
	The system complies with the requirements for electromagnetic compliance (EMC) in Australia and New Zealand.
	The system complies with applicable European directives.

Labels concerning hazardous substances

Label	Meaning
	This symbol indicates that the waste of electrical and electronic equipment must not be disposed as unsorted municipal waste and must be collected separately. Please contact an authorized representative of the manufacturer for information concerning the decommissioning of equipment.
	This symbol indicates that the product contains hazardous materials in excess of the limits established by the Chinese standard SJ/T11363-2006 Requirements for Concentration Limits for Certain Hazardous Substances in Electronics.

2.3 Emergency procedures

This section describes how to do an emergency shutdown of the ÄKTAprime plus system. The section also describes the result in the event of power failure.

Emergency shutdown

In an emergency situation, do as follows to stop the run:

Step	Action
1	To pause the run without ending the method, press the Pause button located at the instrument front.
2	If required, switch off power to the instrument by pressing the Main power switch to the 0 position. The run is interrupted immediately.

Power failure

The result of a power failure depends on which unit that is affected.

Power failure to...	will result in...
ÄKTAprime plus system	<ul style="list-style-type: none">• The run is interrupted immediately, in an undefined state• The data collected up to the time of the power failure is available in PrimeView
Computer	<ul style="list-style-type: none">• The PrimeView computer shuts down in an undefined state• The run continues, but data cannot be saved in PrimeView

2.4 Recycling information

The equipment shall be decontaminated before decommissioning and all local regulations shall be followed with regard to scrapping of the equipment.

Disposal, general instructions

When taking ÄKTAprime plus system out of service, the different materials must be separated and recycled according to national and local environmental regulations.

Recycling of hazardous substances

ÄKTAprime plus instrument contains hazardous substances. Detailed information is available from your GE Healthcare representative.

Disposal of electrical components

Waste of electrical and electronic equipment must not be disposed as unsorted municipal waste and must be collected separately . Please contact an authorized representative of the manufacturer for information concerning the decommissioning of equipment.



3 Installation

ÄKTAprime plus is delivered in protective packing material and shall be unpacked with great care.

Any equipment connected to ÄKTAprime plus must fulfill applicable standards and local regulations.

A video describing the installation process, is supplied with each ÄKTAprime plus system. For detailed information on Installation, see *ÄKTAprime plus Installation Guide*.

3.1 Site requirements

Parameter	Requirement
Electrical power	100-240 V, 50-60 Hz
Ambient temperature	4°C to 40°C
Placement	Stable laboratory bench e.g. 120 × 80 cm
Humidity	20 to 95%, non-condensing

3.2 Transport

The equipment can be transported on a trolley capable of supporting at least 20 kg.



NOTICE

Lift the system in the upright position. Do not use the fractionation arm as a lifting handle.

- Before moving the system:
- disconnect all cables and tubing connected to peripheral components and liquid containers.
 - remove any loose items from the top of the system.
 - grasp the system firmly by placing the fingers under the base of the main unit and lift.
- For more information on Transport, see *ÄKTAprime plus Installation Guide*.

3.3 Unpacking

- Check the equipment for damage before starting assembly and installation.
- Document any damage and contact your local GE Healthcare representative.

Remove straps and packing material and stand equipment upright before starting installation.

3.4 Connections

Communication

Connect the system according to the electrical drawings in [Appendix A Electrical and communication connections, on page 57](#).

Ensure that PrimeView software is installed on the computer. (Only if PrimeView is used as an evaluation system).

Flow path

All parts and tubing are mounted on the system at delivery. Connect a waste tube, buffer and sample bottles, and optional accessories.

Electrical power

Connect the power cord to a grounded power outlet specified in [Section 3.1 Site requirements, on page 26](#).

3.5 Spare parts and accessories

For correct up to date information on spare parts and accessories visit:
www.gelifesciences.com/AKTA

4 Operation

This chapter provides instructions for the use of ÄKTAprime plus.

4.1 Operation overview

Workflow

The typical workflow in ÄKTAprime plus, after turning on the system, can be divided into a number of steps.

Step	Action	Section
1	Prepare the system for a run	Section 4.3 Preparations before start, on page 29
2	Start a run using a method	Section 4.4 Performing a run, on page 34
3	During a run - view and change parameters	Viewing the run, on page 35
4	Procedures after a run	Section 4.5 Procedures after a run, on page 36
5	Evaluate the results	See PrimeView user documentation.

Liquid flow path

See [Appendix B Connection diagram - Liquid flow path, on page 58](#) for an illustration of the liquid flow path in ÄKTAprime plus.

4.2 Starting the instrument

If the system is not already turned on:

- 1 Turn on the system using the **Power** switch at the rear panel. The system now performs a self-test.
- 2 First the system name and software version number are displayed. Several messages are then shown during the self-test. If an error is detected during the self-test, an error message is shown.
- 3 All parameters are automatically set to factory default values during the self-test.
- 4 The self-test takes about 30–40 seconds. When the test is completed, the display shows the **Templates** menu.

Note: The system can be used for most applications after 15 min of lamp warm-up but the full specifications are not obtained until after 1 hour.

4.3 Preparations before start

Buffer preparation

Prepare buffers according to ÄKTAprime plus cue cards.

Sample preparation

- 1 Adjust the sample composition to the binding buffer by:
 - diluting the sample in binding buffer, or
 - buffer exchange using HiTrap™ Desalting or HiPrep™ 26/10 Desalting column.
 - 2 Filter the sample through a 0.45 µm filter.
-

Purification setup



Removing storage solution from the flow path

At delivery and during storage, the flow path is filled with 20% ethanol. This should be removed before continuing the setup.

Note: Do not use buffer with high salt concentration to flush out the ethanol. It might cause too high backpressure.

To flush out the ethanol using deionized water:

- 1 Put the inlet tubing **A1–A8** that is used and **B** in deionized water.

Note: At delivery, only **A1** and **B** are installed.
- 2 Put all waste capillaries, **W1–W3**, in waste.
- 3 Select **Templates** in the main menu using the  and  buttons and press **OK**.
- 4 Select **Application Template** and press **OK**.
- 5 Select **System Wash Method** and press **OK**.
- 6 Choose to wash the **A2–A8** inlet tubing that is used by pressing **OK** at those cursor positions. **A1** and **B** will always be washed.

Note: At delivery, only **A1** and **B** are installed.
- 7 Scroll to **OK** and press the **OK** button.

4 Operation

4.3 Preparations before start

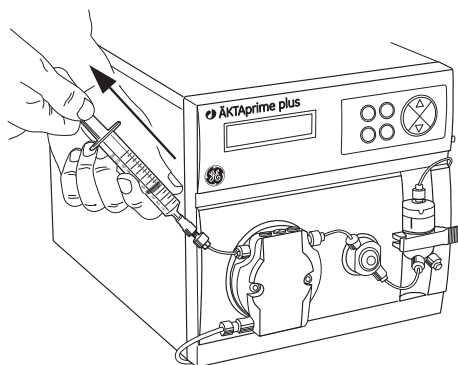
- 8 Press **OK** to start the method.
- 9 When the method is finished, replace the first collection tube. It will contain a small amount of water after the system wash.

Purging the pump and inlet tubing

If there are large amounts of air in the tubing or if you suspect air in the pump, use the Purge kit to purge the flow path. Air bubbles that still are trapped in the pump (causing increased pulsation) can be removed by flushing 100% ethanol through the pump. These two procedures are described in the following two sections.

Purging the flow path using the Purge kit:

- 1 Remove the stop plug from the pump.
- 2 Connect the Purge kit to the pump.



- 3 Put the used inlet tubing in the appropriate buffers.
- 4 Run the pump at 0.1 ml/min.

Filling inlet tubing **A1–A8**:

- 1 Go to **Set Buffer Valve** using the arrow buttons.
- 2 Set the chosen **A** inlet and press **OK**. The valve switches to the selected port.
- 3 Draw buffer with the purge syringe until liquid enters the syringe.
- 4 Repeat step 1–3 until all chosen **A** inlet tubing is filled.

Filling inlet tubing **B**:

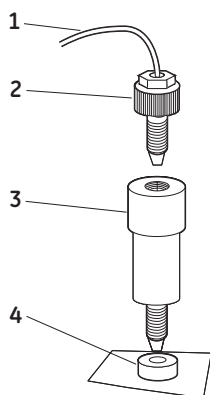
- 1 Go to **Set Concentration %B** and set the concentration to **100%**.
- 2 Press **OK**. The switch valve turns to the inlet **B** port.
- 3 Draw buffer with the purge syringe until liquid enters the syringe.
- 4 Replace the purge tubing with the stop plug.
- 5 Stop the pump by pressing **end** and then **OK**.

Flushing the pump with 100% ethanol:

- 1 Put inlet tubing **A1** in deionized water.
- 2 Run the pump at 40 ml/min for 1 min and press **pause/cont**.
- 3 Move inlet tubing **A1** to 100% ethanol
- 4 Press **pause/cont**, run the pump for 10–20 s and press **pause/cont**.
- 5 Set the flow rate to 5 ml/min using the arrow buttons.
- 6 Press **pause/cont**, run the pump for at least 30 s and press **pause/cont**.
- 7 Move inlet tubing **A1** to deionized water.
- 8 Press **pause/cont** and run the pump for 1 min.
- 9 Finish by pressing **end** and then **OK**.

Preparing the tubing and column

- 1 Put inlet tubing **A1** in the binding buffer.
- 2 Put inlet tubing **B** in the elution buffer.
- 3 Put the three waste capillaries (brown color) from port **4** and **5** on the injection valve and port **NO** on the fraction collector valve in waste.
- 4 Connect a column, for example a HisTrap™ HP 1 ml column, between port **1** on the injection valve and the upper port of the UV flow cell. Use a suitable length of PEEK tubing and 1/16" male connectors.



No.	Description	No.	Description
1	From injection valve	3	HisTrap column
2	1/16" male connector	4	UV cell

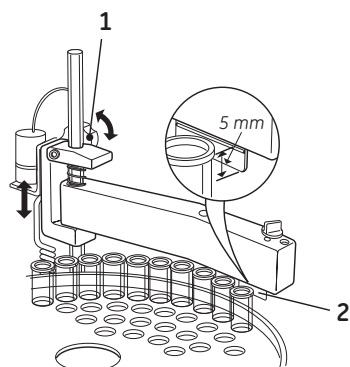
Note: Other unions and connectors might be required for other columns.

4 Operation

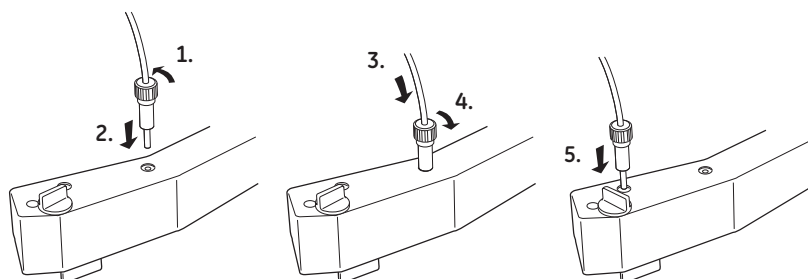
4.3 Preparations before start

Preparing the fraction collector

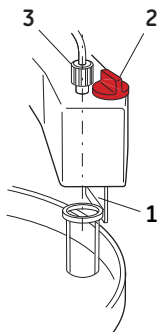
- 1 Fill the fraction collector rack with, for example, 18 mm tubes (minimum 40 pcs.).
- 2 Adjust the height of the delivery arm using the lock knob (1) so that the bottom of the tube sensor (2) is about 5 mm below the top of the tubes. The tubes should always be below the horizontal mark on the tube sensor.



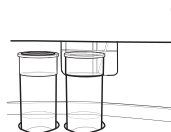
- 3 If necessary, adjust the length of the tubing exposed according to the sequence shown below (the hole in the delivery arm used in step 3 and 4 is only used for adjusting the tubing length).



- 4 Check that the tube sensor (1) is in the correct position for the tube size. The eluent tubing should be over the center of the collection tube. Use the red sensor control knob (2) to position the tube holder (3).



- 5 Rotate the rack by hand until the rear half of the tube sensor rests against the first tube.



- 6 Press **feed tube** on the front panel (see [Figure 1.2](#)). The bowl moves to the correct position to collect the first fraction in the first tube.
- 7 Make sure that drop synchronization is turned on.

Note: Drop synchronization can NOT be used at flowrates above 3 ml/min.

Preparing the monitors

- 1 Check the UV lamp filter position and the lamp position.
- 2 Calibrate the pH electrode (optional).

See *ÄKTAprime plus User Manual* for more information.

Filling the buffer inlet tubing

When running an application templates, the buffer inlet tubing will automatically be filled with buffer.

For other applications, fill the inlet tubing manually with buffer as described in the *ÄKTAprime plus User Manual*.

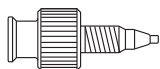
Filling the sample loop

Using an injection fill port

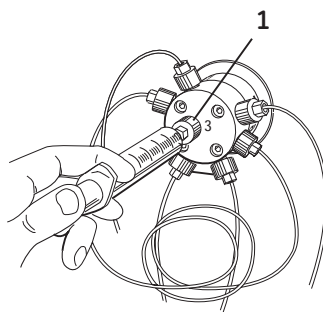
4 Operation

4.3 Preparations before start

- 1 Connect a sample loop between port **2** and **6** on the injection valve. Make sure that the sample loop is large enough for your sample.
- 2 Connect a luer female/1/16" male union to port **3**.



- 3 Fill a syringe with five loop volumes of deionized water or binding buffer.
- 4 Fit the syringe in the Luer union (1) and carefully inject the buffer.



- 5 Remove the syringe and fill it with at least two loop volumes of the sample.
- 6 Carefully inject the sample into the sample loop. Do NOT remove the syringe after the injection because the loop might otherwise be emptied due to self-drainage or air may be introduced in the flow path.

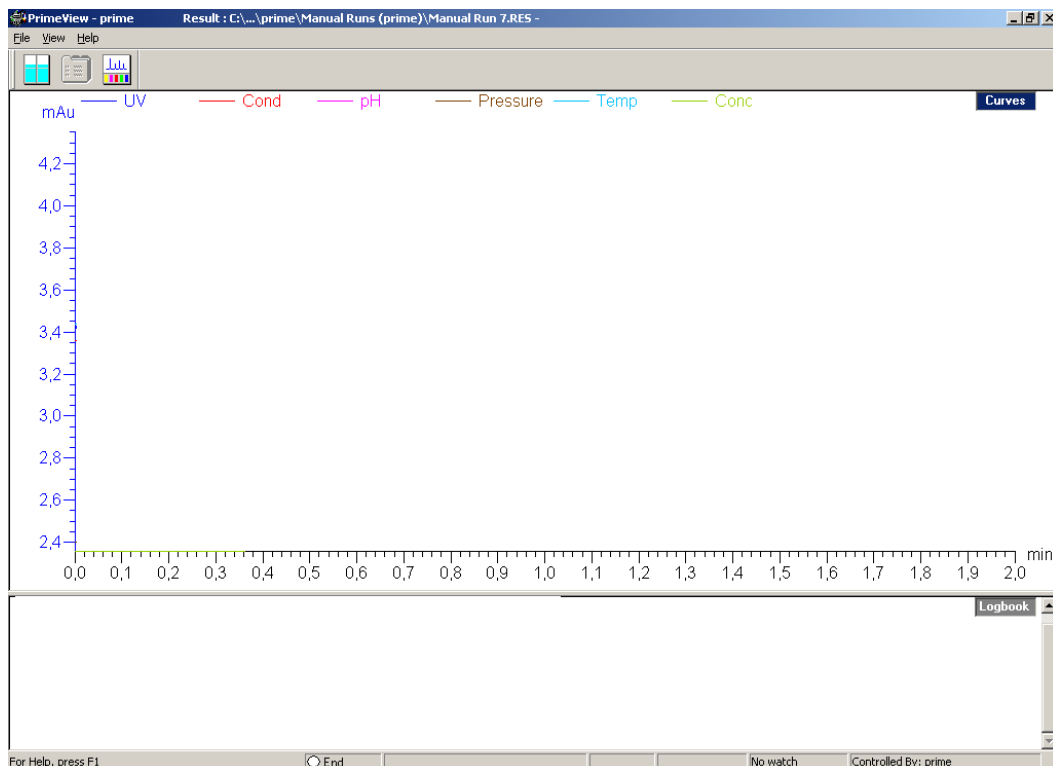
4.4 Performing a run

Selecting template and starting the run

- 1 Select **Templates** in the main menu and press **OK**.
- 2 Select **Application Template** and press **OK**.
- 3 Select the appropriate template, for example **His Tag Purification HisTrap**, and press **OK**.
- 4 Set the sample volume and press **OK**.
- 5 Press **OK** to start the purification run.

Viewing the run

When the pump starts running, the progress of the run can be viewed in the two panes in PrimeView.



- The **Curves** pane displays monitor signal values graphically.
- The **Logbook** pane displays all actions (e.g. method start and end, base instructions and method instructions) and unexpected conditions (e.g. warnings and alarms). The log is saved in the result file.

Selecting curves to be displayed

- 1 In PrimeView module, select **View:Properties**.
- 2 In the **Properties** dialog, click the **Curves** tab.
- 3 In the **Display curves** list, select the curves you want to display.
- 4 Click **OK**.

For more information on customizing the view panes, see *PrimeView User Manual*.

Ending the run

Press **OK** at the **Method Complete** prompt. This will cause all valves to return to their default positions.

To stop the run on a system before it is finished:

- 1 Press the **end** button.
 - 2 Select **yes** and press **OK**.
-

Error indication

When a warning or an alarm is issued from a system, an error code is displayed. See *ÄKTAprius plus User Manual* for guidance.

Evaluate the results

PrimeView Evaluation module provides facilities for the presentation and evaluation of separation results.

To start **PrimeView Evaluation** module, click **PrimeView Evaluation** icon on the Windows desktop.



See *ÄKTAprius plus User Manual* and *PrimeView User Manual* for how to evaluate the results.

4.5 Procedures after a run

Cleaning after a run



NOTICE

Do not allow solutions which contain dissolved salts, proteins or other solid solutes to dry out in the UV flow cell.



NOTICE

Do not allow particles to enter the UV flow cell as damage to the flow cell might occur.

Buffers not containing any salt can be left in the system for a short time after a run, even overnight (not in the pH electrode, see instructions below).



NOTICE

If a buffer containing salt has been used, the flow path must be flushed with deionized water.

To flush the flow path:

- 1 Fill a syringe with five times the sample loop volume of deionized water.
- 2 Rinse the sample loop by injecting the water through the fill port on the injection valve.
- 3 Put all used inlet tubings in water.
- 4 In the **Templates** menu, select **Application Template** and then **System Wash Method**.
- 5 Select the used inlet ports. Inlets **A1** and **B** will always be washed.
- 6 Press **OK** to start the method. The system flow path is now automatically flushed.

For information on cleaning and long-term storage, see [Section 5.3 Cleaning, on page 41](#) and [Section 5.7 Storage, on page 44](#).

5 Maintenance

This chapter provides instructions for routine component maintenance and a maintenance schedule.

5.1 General

Regular maintenance is important for safe and trouble-free operation of your instrument. The user should perform daily and monthly maintenance. Preventive maintenance should be performed on a yearly basis by qualified service personnel.

For maintenance of a specific component, carefully read the component manual and follow the instructions. To avoid personal injury when performing maintenance on the ÄKTaprime plus instrument, follow the instructions below.



WARNING

Electrical shock hazard. All repairs should be done by service personnel authorized by GE Healthcare. Do not open any covers or replace parts unless specifically stated in the user documentation.



WARNING

Disconnect power. Always disconnect power from the instrument before replacing any component on the instrument, unless stated otherwise in the user documentation.



WARNING

Hazardous chemicals during maintenance. When using hazardous chemicals for system or column cleaning, wash the system or columns with a neutral solution in the last phase or step.



WARNING

Do not perform any type of maintenance work while the system is powered electrically or when the piping system is pressurized. Note that the piping system can be pressurized even when the system is closed down.



CAUTION

Fire hazard. Follow instructions in the ÄKTAprime plus User Manual for correct installation of a new UV-lamp. If the lamp is not installed properly it may be overheated and cause a fire hazard.



NOTICE

When using hazardous chemicals, take all suitable protective measures, such as wearing protective glasses and gloves resistant to the chemicals used. Follow local regulations and instructions for safe operation and maintenance of the system.



NOTICE

Cleaning. Keep the instrument dry and clean. Wipe regularly with a soft damp tissue and, if necessary, a mild cleaning agent. Let the instrument dry completely before use.

5.2 User maintenance schedule

[Table 5.1](#) provides a guide to maintenance operations and intervals at which these operations should be performed by the user. The user is however responsible for deciding the type of operations and length of intervals necessary to maintain system function and safety.

Table 5.1: User maintenance schedule

Interval	Action	Instructions/reference
Daily	Leak inspection	Visually inspect the system for leaks.
	Wash the system flow path	<ol style="list-style-type: none"> 1 For cleaning the flow path, see Cleaning-In-Place, on page 41. 2 For leaving the system for a few days, see Section 5.7 Storage, on page 44.
	Calibrate pH electrode (optional)	Calibrate the pH electrode (if applicable) according to <i>Monitor pH/C-900 User Manual</i> .
Weekly	Check inlet filters	Check the inlet filters visually and replace them if necessary.
	Replace on-line filter (if applicable)	Replace the on-line filter.
Monthly	Flow restrictor	<p>Check that flow restrictor generates the following back-pressure:</p> <p>FR-904: 0.4 ±0.05 MPa</p> <p>FR-902: 0.2 ±0.05 MPa</p> <p>Check the back-pressure as follows:</p> <ol style="list-style-type: none"> 1 Disconnect the flow restrictor. 2 Connect a capillary (approx. 1 m, i.d. 1 mm) to the waste port (port 5) on the injection valve. Set the injection valve manually to Waste position. Put the open end in a waste container. 3 Run the pump manually at 10 ml/min with water. Note the back-pressure (Bp1) on the pump display, or in the Run Data window. 4 Set the system to Pause and connect the flow restrictor to the open end of the capillary (observe the IN marking). Put the flow restrictor in the waste container. 5 Press Continue so that the pump run at 10 ml/min with water. Note the back-pressure (Bp2) on the pump display, or in the Run Data window. 6 Calculate the back-pressure generated by the flow restrictor (Bp2-Bp1). Replace it if it is not within limit.

Interval	Action	Instructions/reference
Yearly	Valve inspection	Check for external or internal leakage. Replace channel plate and distribution plate yearly or when required. Refer to the relevant valve instruction sheet.

5.3 Cleaning

Cleaning before maintenance/service

Before maintenance/service is performed, the system owner must first clean the system and complete a Decontamination Report. Contact GE Healthcare for further information.

Cleaning-In-Place

All components in the system are designed for ease of CIP.

After repeated separation cycles, contaminating material might progressively build up in the system and on the column. This material may not have been removed by the cleaning step described above. The nature and degree of contamination depends on the sample and the chromatographic conditions employed. These should be considered when designing a cleaning protocol.

Routine cleaning should be performed at intervals aimed at prevention rather than cleaning the system from growth or contamination.



WARNING

Make sure that the piping system is completely leakage free before performing any CIP on the system.

Make sure that the process control method for cleaning flushes all possible flow paths in the system. After cleaning, rinse the entire system with water or suitable liquid until the piping/tubing system is completely free from the CIP solution (monitors in the system can be used as detectors). Do not leave NaOH or other cleaning agents in the system for long periods.



WARNING

Hazardous chemicals during maintenance. When using hazardous chemicals for system or column cleaning, wash the system or columns with a neutral solution in the last phase or step.



WARNING

NaOH is corrosive and therefore dangerous to health. When using hazardous chemicals, avoid spillage and wear protective glasses and other suitable personal protective equipment.

See also [Section 5.7 Storage, on page 44](#)

5.4 Component maintenance

Maintenance and preventive replacement of parts of the major components are described in the respective manuals included in the system documentation.

The system documentation also includes a spare part list to be used to find common spare parts and their code numbers for ordering. This list can also be found online at www.gelifesciences.com/AKTA.

5.5 Disassembly and assembly of components and consumables

The operator must carefully read and understand the instructions supplied for each component before disassembly and assembly of the component. When replacing consumables, such as tubing and tubing connectors, all necessary safety precautions must be taken. Contact your local GE Healthcare representative if further information or help is needed.



WARNING

Disconnect power. Always disconnect power from the instrument before replacing any component on the instrument, unless stated otherwise in the user documentation.



WARNING

Before disassembly, check that there is no pressure in the piping system.



WARNING

After assembly, the piping system must be tested for leakage at maximum pressure for continued protection against injury risks due to fluid jets, burst pipes or explosive atmosphere.

5.6 Calibration

The table below lists the type and frequency of calibrations that can be done on the instrument. Refer to PrimeView user documentation and to the individual component User Manuals and Instructions for descriptions of how to perform these calibrations. The calibrations are performed from PrimeView by selecting **System:Calibrate** in **System Control**.

Component		How often
pH monitor (if applicable)		Every day.
Pump (if applicable)		When required.
Pressure reading		When required.
Conductivity flow cell	Cell constant	Only necessary if specific conductivity with high accuracy is measured (Cond_Calib).
	Temperature	Must be done when changing the conductivity flow cell (Temp).
	Entering a new cell constant	Must be done when changing the conductivity flow cell (Cond_Cell).

5.7 Storage

General recommendation

For storage, the system must first be cleaned as described in [Cleaning-In-Place, on page 41](#). After cleaning, the system must be filled with 0.01 M NaOH or 20% ethanol solution.

Columns and media shall be stored according to their respective instructions.

Storage conditions

The following conditions shall be maintained while the system is in storage:

- Temperature: +2 to +30°C (preferably room temperature)
- Relative humidity: 0 to 95%, non-condensing (preferably low humidity).

After storage, clean the system, calibrate all monitors, and perform a leakage test before using the system.

6 Troubleshooting

6.1 UV curve problems

Error symptom	Possible cause	Corrective action
Ghost peak	Dirt or residues in the flow path from previous runs. Air in the eluents.	Clean the system. Make sure air is removed.
	Residue in the column from previous runs	Clean the column according to the column instructions.
	Incorrect mixer function	Check the mixer function by placing a stirrer bar on top of the mixer housing. The stirrer bar should rotate when the system is in Run mode. The mixer function can also be checked by running the installation test.
Noisy UV-signal, signal drift or instability	Dirty UV cell	Clean the UV cell by flushing Deconex 90, Deconex 11 or equivalent.
	Impure buffer	Check if the signal is still noisy with water.
	Air in the pump or in the UV cell	Purge the pump according to Pump User Manual. Run a system wash with buffer.

6 Troubleshooting

6.1 UV curve problems

Error symptom	Possible cause	Corrective action
Low sensitivity	Aging UV lamp	Check the lamp run time according to and replace if necessary. Refer to <i>ÄKTApriime plus User Manual</i> .
	UV lamp in wrong position	Check that the lamp position and the filter position are both set to the wavelength to be used, 280 nm or 254 nm. Refer to <i>ÄKTApriime plus User Manual</i> .
	The theoretical extinction coefficient too low	Calculate the theoretical extinction coefficient of the protein. If it is zero or very low at 280 nm, the protein cannot be detected.

6.2 Conductivity curve problems

Error symptom	Possible cause	Corrective action
Baseline drift or noisy signal	Air in the pump or the flow cell	Use a flow restrictor after the flow cell.
	Leaking tube connections	Tighten the clamps. If necessary, replace the clamps.
	Incorrect mixer function	Check the mixer function by placing a stirrer bar on top of the mixer housing. The stirrer bar should rotate when the system is in Run mode. The mixer function can also be checked by running the installation test.
	Dirty conductivity cell	Clean the conductivity cell by flushing 1 M NaOH or 20% ethanol.
	Column not equilibrated	Equilibrate the column. If necessary, clean the column using a method plan for column cleaning.
Conductivity measurement with the same buffer appears to decrease over time	Dirty flow cell	Clean the flow cell according to procedure in Monitor User Manual.
	Decrease in ambient temperature	Use a temperature compensation factor. See Monitor User Manual.

Error symptom	Possible cause	Corrective action
Waves on the gradient	Incorrect pump function	Check that the pump is operating and is programmed correctly.
	Dirty mixing chamber	Check that the mixing chamber is free from dirt or particles.
	Insufficient mixing chamber volume	Change to a larger mixing chamber volume if necessary.
	Incorrect motor function	Check the motor operation. Place a hand on the mixer and start it by starting the pump at a low flow rate. You should both hear and feel the mixer motor and stirrer when they are spinning.
Ghost peaks appear in the gradient profile	Air in the flow cell	Check for loose tubing connections. Use the flow restrictor.
Unlinear gradients or slow response to %B changes	Dirty tubing	Wash the tubing and check pump is operating properly.
	Incorrect mixer volume	Change to smaller mixer volume.

Error symptom	Possible cause	Corrective action
Incorrect or unstable reading	Loose connection of conductivity flow cable	Check that the conductivity flow cell cable is connected properly to the rear of the instrument.
	Incorrect pump and valves function	Check that the pump and valves operate correctly.
	Incorrect temperature compensation factor	If temperature compensation is being used, check that the temperature sensor is calibrated, and that the correct temperature compensation factor is in use.
	Dirty or incorrectly calibrated column	Check that the column is equilibrated. If necessary clean the column.
	Incorrect mixer function	Check the operation of the mixer. The mixer function is checked by placing a stirrer bar on top of the mixer housing. The stirrer bar should rotate when the system is in Run mode. The mixer function can also be checked by running the installation test.

6.3 pH curve problems

Error symptom	Possible cause	Corrective action
No response to pH changes	Faulty electrode connection	Check that the electrode cable is connected properly to the rear of the instrument.
	Damaged electrode	The electrode glass membrane may be cracked. Replace the electrode.
	Incorrectly connected pH monitor	Check that the pH monitor is correctly connected according to the User Manual.
Small response to pH changes	Dirty pH electrode	Clean the pH electrode as detailed in <i>Monitor pH/C-900 User Manual</i> . If the problem remains, replace the pH electrode.

Error symptom	Possible cause	Corrective action
Slow pH response or Calibration impossible	Contaminated electrode glass membrane	Check the electrode glass membrane. If it is contaminated, clean the electrode following the instructions in <i>Monitor pH/C-900 User Manual</i> .
	Membrane has dried out	If the membrane has dried out, the electrode may be restored by soaking it in buffer overnight.

Error symptom	Possible cause	Corrective action
Incorrect or unstable pH reading	Problem with electrode	<p>Check that the electrode cable is connected properly to the rear of the instrument.</p> <p>Check that the electrode is correctly inserted in the flow cell and, if necessary, hand-tighten the nut.</p> <p>Check that the pH electrode is not broken.</p> <p>Calibrate the pH electrode.</p> <p>Clean the pH electrode if required, see <i>Monitor pH/C-900 User Manual</i>.</p> <p>Compare the response of the pH electrode with that of another pH electrode. If the response differ greatly, the electrode may require cleaning or replacement.</p> <p>In organic solvents such as ethanol, methanol and acetonitrile, stable pH measurements are not possible since dehydration of the membrane will occur. It is recommended that the pH electrode is not used in applications using organic solvents. Mount the dummy electrode instead.</p>
	Incorrect pump or valve operation	Check that the pump and valves operate correctly.
	Air in the flow cell	If air in the flow cell is suspected, tap the flow cell carefully or tilt it to remove the air. Alternatively, flush the cell with buffer at 20 ml/min (E 100 system) or 10 ml/min (E 10 system) for 1/2 min. Use the flow restrictor FR-902 after the pH electrode.
	Static interference	There may be interference from static fields. Connect the pH flow cell and the rear panel of the monitor using a standard laboratory 4 mm "banana plug" cable.

Error symptom	Possible cause	Corrective action
pH values vary with varied back pressure	Problem with the electrode	Replace the pH electrode.

6.4 Pressure curve problems

Error symptom	Possible cause	Corrective action
Erratic flow, noisy baseline signal, irregular pressure trace	Air bubbles passing through or trapped in the pump	Check all connections for leaks. Check that there is sufficient eluent present in the reservoirs. Use degassed solutions. Purge the pump. Follow the instructions in <i>ÄKTApri^{me} plus User Manual</i> .
	Inlet or outlet check valves not functioning correctly	Clean the valves in according to <i>ÄKTApri^{me} plus User Manual</i> .
	Piston seal leaking	Replace the piston seal according to the instructions in <i>ÄKTApri^{me} plus User Manual</i> .
	Blockage or part blockage of flowpath	Flush through to clear blockage. If necessary, replace tubing. Check inlet tubing filter. It can become clogged if unfiltered buffers or samples are applied. See instructions for flushing through at the end of the run in <i>ÄKTApri^{me} plus User Manual</i> .

7 Reference information

This chapter contains technical data, regulatory and other information.

7.1 Specifications

Parameter	Value
Ingression protection	Housing: IP20 Flow cells: IP44
Supply voltage	100-120/220-240 V ~, 50 to 60 Hz
Power consumption	90 VA
Fuse specification	T 1.0 AH 250 V
Dimensions (H × W × D)	530 × 400 × 450 mm
Weight	13 kg
Ambient temperature	+4 to +40 °C
Relative humidity tolerance (non-condensing)	10 to 95%
Atmospheric pressure	84 to 106 kPa (840 to 1060 mbar)

7.2 Chemical resistance

Chemical	Exposure < 1 day	Exposure up to 2 months	CAS no.	EEC no.	Comments
Acetaldehyde	OK	OK			
Acetic acid, < 5%	OK	OK			
Acetic acid, 70%	OK	OK	64-19-7	200-580-7	
Acetonitrile	OK	OK	75-05-8	200-835-2	FFKM, PP and PE swell.
Acetone, 10%	OK	Avoid			PVDF is affected by long term use.

Chemical	Exposure < 1 day	Exposure up to 2 months	CAS no.	EEC no.	Comments
Ammonia, 30%	OK	OK	7664-41-7	231-635-3	Silicone is affected by long-term use.
Ammonium chloride	OK	OK	12125-02-9	235-186-4	
Ammonium bicarbonate	OK	OK			
Ammonium nitrate	OK	OK			
Ammonium sulphate	OK	OK	7783-20-2	231-984-1	
1-Butanol	OK	OK			
2-Butanol	OK	OK			
Citric acid	OK	OK	29340-81-6	249-576-7	
Chloroform	OK	Avoid			Kalrez™, CTFE, PP and PE are affected by long term use.
Cyclohexane	OK	OK			
Detergents	OK	OK			
Dimethyl sulphoxide	Avoid	Avoid	67-68-5	200-664-3	PVDF is affected by long term use.
1, 4-Dioxane	Avoid	Avoid			ETFE, PP, PE and PVDF are affected by long term use.
Ethanol, 100%	OK	OK	75-08-1	200-837-3	
Ethyl acetate	OK	Avoid			Silicone not resistant. Pressure limit for PEEK decreases.
Ethylene glycol, 100%	OK	OK	107-21-1	203-473-3	
Formic acid, 100%	OK	OK	64-18-6	200-579-1	Silicone not resistant.

7 Reference information

7.2 Chemical resistance

Chemical	Exposure < 1 day	Exposure up to 2 months	CAS no.	EEC no.	Comments
Glycerol, 100%	OK	OK	56-81-5	200-289-5	
Guanidinium hydrochloride	OK	OK			
Hexane	OK	Avoid			Silicone not resistant. Pressure limit for PEEK decreases.
Hydrochloric acid, 0.1 M	OK	OK	7647-01-0	231-595-7	Silicone not resistant.
Hydrochloric acid, > 0.1 M	OK	Avoid			Silicone not resistant. Titanium is affected by long term use.
Isopropanol, 100%	OK	OK	67-63-0	200-661-7	
Methanol, 100%	OK	OK	74-93-1	200-659-6	
Nitric acid, diluted	OK	Avoid			Silicone not resistant.
Nitric acid, 30%	Avoid	Avoid			Elgiloy is affected by long term use.
Phosphoric acid, 10%	OK	Avoid	7664-38-2	231-633-2	Titanium, aluminium oxide and glass are affected by long term use.
Potassium carbonate	OK	OK	584-08-7	209-529-3	
Potassium chloride	OK	OK	7447-40-7	231-211-8	
Pyridine	Avoid	Avoid			ETFE, PP and PE not resistant.
Sodium acetate	OK	OK			
Sodium bicarbonate	OK	OK			
Sodium bisulphate	OK	OK			

Chemical	Exposure < 1 day	Exposure up to 2 months	CAS no.	EEC no.	Comments
Sodium borate	OK	OK			
Sodium carbonate	OK	OK			
Sodium chloride	OK	OK	7647-14-5	231-598-3	
Sodium hydroxide, 2 M	OK	Avoid	1310-73-2	215-185-5	PVDF and borosili- cate glass are af- fected by long term use.
Sodium sulphate	OK	OK	7757-82-6	231-820-9	
Sulphuric acid, dilut- ed	OK	Avoid			PEEK and titanium are affected by long term use.
Sulphuric acid, medium concentra- tion	Avoid	Avoid			
Tetrachloroethy- lene	Avoid	Avoid			Silicone, PP and PE are not resistant.
Tetrahydrofuran	Avoid	Avoid			ETFE, CTFE, PP and PE are not resistant.
Toluene	OK	Avoid			Pressure limit for PEEK decreases.
Trichloroacetic acid, 1%	OK	OK	76-03-9	200-927-2	
Trifluoroacetic acid, 1%	OK	OK	176-05-1	200-929-3	
Urea, 8M	OK	OK	57-13-6	200-315-5	
o-Xylene and p-Xy- lene	OK	Avoid			PP and PE are af- fected by long term use.

7.3 System recommendations

Refer to *ÄKTAprius plus User Manual*, or contact your local GE Healthcare representative for the most current information.

7.4 Literature

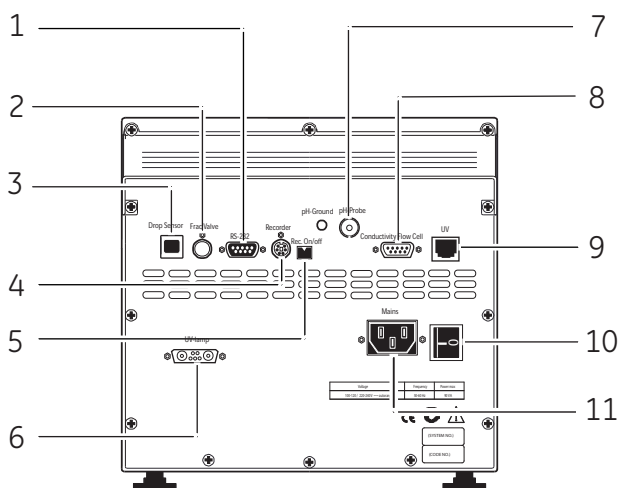
For further information regarding the ÄKTAprime plus system, refer to the following:

- *ÄKTAprime plus User Manual*
- *ÄKTAprime plus Installation Guide*
- *PrimeView User Manual*

7.5 Ordering information

For ordering information visit www.gelifesciences.com/AKTA.

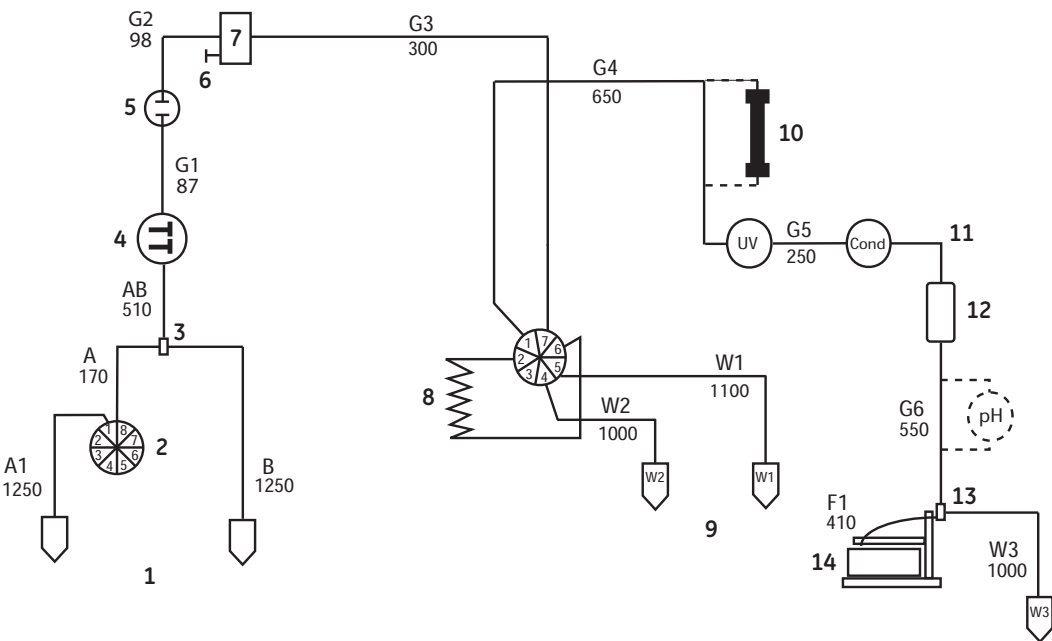
Appendix A Electrical and communication connections



No.	Connection	No.	Connection
1	RS-232 to computer	7	pH electrode
2	Flow diversion valve	8	Conductivity flow cell
3	Fraction collector	9	Optical unit
4	Measurement data to recorder	10	Power switch
5	On/off signals to recorder	11	Power inlet
6	UV lamp		

Figure A.1: Electrical and communication connections for ÄKTAprius plus.

Appendix B Connection diagram - Liquid flow path



No.	Description	No.	Description
1	Buffers	8	Loop (500 µl)
2	Buffer valve	9	Waste
3	Gradient switch valve	10	Column
4	System pump	11	Male/Male
5	Pressure monitor	12	Flow restrictor (0.2 MPa)
6	Stop plug	13	Flow diversion valve
7	Mixer	14	Fraction collector

Appendix C Tubing

Names in the Label column in [Table C.1](#) refer to tubing labels in the liquid flow path connection diagram, see [Appendix B Connection diagram - Liquid flow path, on page 58](#).

Table C.1: Tubing specifications for ÄKTAprime plus

Use	Label	Material	Length (mm)	I.D. (mm)	Volume (µl)
Inlet A11	A1	Teflon™	1250	2.9	8.2×10^3
Inlet A1	A	Teflon	170	2.9	1.1×10^3
Inlet A2	B	Teflon	1250	2.9	8.2×10^3
Switch valve - Pump A3	AB	Teflon	510	1.6	1.0×10^3
Pump - Pressure monitor	G1	PEEK	150	0.75	66
Pressure monitor - Mixer	G2	PEEK	120	0.75	53
Mixer - Valve	G3	PEEK	300	0.75	133
Valve - UV (Column)	G4	PEEK	650	0.75	287
UV - Cond	G5	PEEK	250	0.75	110
Cond - Flow restrictor	Union, 1/16" male / 1/16" male	PEEK	38	0.50	7
Flow restrictor - Frac. coll.	G6	PEEK	550	0.75	243
Frac. tubing	F1	PEEK	410	0.75	181
Waste	W1, W2, W3	PEEK	1000	1.0	785

For local office contact information, visit
www.gelifesciences.com/contact

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